

# UC San Diego

## UC San Diego Previously Published Works

### Title

Biomarkers of Inflammation and Fibrosis in Kawasaki Disease Patients Years After Initial Presentation With Low Ejection Fraction.

### Permalink

<https://escholarship.org/uc/item/30b995sq>

### Journal

Journal of the American Heart Association, 9(1)

### ISSN

2047-9980

### Authors

Hoshino, Shinsuke  
Shimizu, Chisato  
Jain, Sonia  
et al.

### Publication Date

2020

### DOI

10.1161/jaha.119.014569

Peer reviewed

# Biomarkers of Inflammation and Fibrosis in Kawasaki Disease Patients Years After Initial Presentation With Low Ejection Fraction

Shinsuke Hoshino, MD; Chisato Shimizu, MD; Sonia Jain, PhD; Feng He, MS; Adriana H. Tremoulet, MD, MAS; Jane C. Burns, MD

**Background**—Coronary artery aneurysms and myocarditis are well-recognized complications of Kawasaki disease (KD) but no systematic evaluation of the consequences of myocarditis has been performed in the subset presenting with low ejection fraction (EF). We postulated that more severe myocardial inflammation as evidenced by low EF during the acute phase could lead to late myocardial fibrosis.

**Methods and Results**—We measured the carboxyterminal propeptide of procollagen type I (PIPC), soluble suppressor of tumorigenicity 2, galectin-3 (Gal-3), growth-differentiation factor-15, and calprotectin by ELISA in late convalescent blood samples from 16 KD patients who had an EF  $\leq 55\%$  on their initial echocardiogram. Results were compared with samples from sex- and age-matched KD patients with initial EF  $> 60\%$ . In the univariate analysis, the median Gal-3 and PIPC levels in the low EF group were significantly higher than those in the normal EF group (Gal-3: low EF 6.216 versus normal EF 4.976 mg/dL  $P=0.038$ , PIPC: low EF 427.4 versus normal EF 265.2 mg/dL,  $P=0.01$ ). In a multivariable analysis, there were significant differences for Gal-3 and PIPC levels between the low and normal EF groups, adjusting for age, sex, and worst z score.

**Conclusions**—Convalescent KD patients with a history of low EF during the acute illness had significantly elevated levels of Gal-3 and PIPC when compared with matched-control KD patients with normal EF. These findings raise concern for myocardial fibrosis as a potential late sequela of the more severe myocarditis experienced by a subset of KD patients during the acute phase. (*J Am Heart Assoc.* 2020;9:e014569. DOI: 10.1161/JAHA.119.014569.)

**Key Words:** galectin-3 • Kawasaki disease • myocardial fibrosis • myocarditis • PIPC

Kawasaki Disease (KD) is a self-limited, acute vasculitis of children whose etiology is still unknown.<sup>1</sup> The most significant complications are coronary artery aneurysms (CAA) that occur in 15% to 25% of untreated patients and 5% of patients treated with intravenous immunoglobulin (IVIG).<sup>2</sup> These important cardiovascular lesions may lead to ischemic heart disease, myocardial infarction, and sudden death.<sup>3</sup> However, myocarditis is also an important complication of KD, with subclinical left ventricular (LV) dysfunction occurring in 50% to 70% of patients in the acute phase.<sup>4,5</sup> The myocarditis improves soon after IVIG treatment<sup>6</sup> and

abnormalities of LV function resolve by 1 to 3 years after KD onset.<sup>7,8</sup> The severity of myocarditis is not necessarily predictive of the severity of coronary artery damage. In 2 series of KD patients presenting with shock and decreased ejection fraction, only 15 and 43% of patients developed CAA despite clinical manifestations of myocarditis.<sup>9,10</sup> Endomyocardial biopsies in KD patients performed during the subacute phase demonstrated histological evidence of myocarditis in all patients despite the absence of clinical signs of myocarditis.<sup>11,12</sup> We postulated that patients initially presenting with a low EF as a clinical sign of myocardial inflammation could be at risk for late myocardial fibrosis.

There is controversy regarding how well plasma biomarkers predict the histologic finding of myocardial fibrosis.<sup>13</sup> From the adult heart failure literature, the carboxyterminal propeptide of procollagen type I (PIPC) has been used as a biomarker of myocardial fibrosis.<sup>14</sup> Increased serum PIPC levels correlate with adverse outcomes in heart failure and myocardial infarction.<sup>15,16</sup> Other candidate biomarkers implicated in both myocardial fibrosis and inflammation include soluble suppressor of tumorigenicity 2 (sST2), galectin-3 (Gal-3), growth-differentiation factor-15 (GDF-15), and calprotectin.<sup>14,17</sup> We previously reported that elevated serum levels of sST2, a member of the interleukin-1 receptor family and a

From the Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, CA (S.H., C.S., A.H.T., J.C.B.); Department of Family Medicine and Public Health, University of California San Diego, La Jolla, CA (S.J., F.H.); Rady Children's Hospital San Diego, San Diego, CA (A.H.T., J.C.B.).

**Correspondence to:** Shinsuke Hoshino, MD and Jane C. Burns, MD, University of California San Diego School of Medicine, 9500 Gilman Dr, MC 0641, La Jolla, CA 92093-0641. E-mail: hoshinos@belle.shiga-med.ac.jp; jcburns@ucsd.edu

Received September 10, 2019; accepted November 15, 2019.

© 2019 The Authors. Published on behalf of the American Heart Association, Inc., by Wiley. This is an open access article under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

## Clinical Perspective

### What Is New?

- We measured biomarkers of inflammation and fibrosis in pediatric patients with Kawasaki disease who initially presented with low (<55%) ejection fraction but with normal or mildly dilated coronary arteries.
- Although the ejection fraction normalized in all patients after treatment, biomarkers of fibrosis were elevated years after the initial illness.
- No long-term study has previously evaluated markers of myocardial fibrosis in this subset of Kawasaki disease patients with initial reduced ejection fraction.

### What Are the Clinical Implications?

- The finding of elevated biomarkers of fibrosis in Kawasaki disease patients who initially presented with severe myocarditis and reduced ejection fraction raises concern for late myocardial fibrosis, similar to what has been noted on adult autopsy studies of Kawasaki disease patients.
- This subset of Kawasaki disease patients may warrant long-term follow-up and advanced imaging studies to evaluate for potential myocardial fibrosis, although this is not recommended in the current American Heart Association guidelines.

decoy receptor for interleukin-33, are correlated with impaired myocardial relaxation in acute KD subjects.<sup>18</sup> Gal-3 is a  $\beta$ -galactoside-binding lectin that plays an important role in inflammation, fibrosis, and cell differentiation<sup>19</sup> and plasma Gal-3 levels are higher in KD subjects with giant coronary artery aneurysms (CAA) compared with KD subjects without aneurysms and healthy controls.<sup>20</sup> GDF-15 is a member of the TGF- $\beta$  superfamily and it is not normally expressed in the myocardium but is strongly upregulated in cardiomyocytes by various stressors, including proinflammatory cytokines.<sup>21</sup> We recently reported molecular evidence of on-going inflammation by shotgun proteomics in late convalescent pediatric and adult KD patients with giant CAA.<sup>17</sup> These groups showed increased levels of calprotectin, a marker of inflammation secreted by neutrophils and monocytes. In the present study, we sought to determine if pediatric KD patients with low EF during their acute illness have evidence of myocardial inflammation and fibrosis as evidenced by elevated levels of our candidate biomarkers at least 1 year after disease onset.

## Methods

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study materials will not be made available because of limited availability of samples.

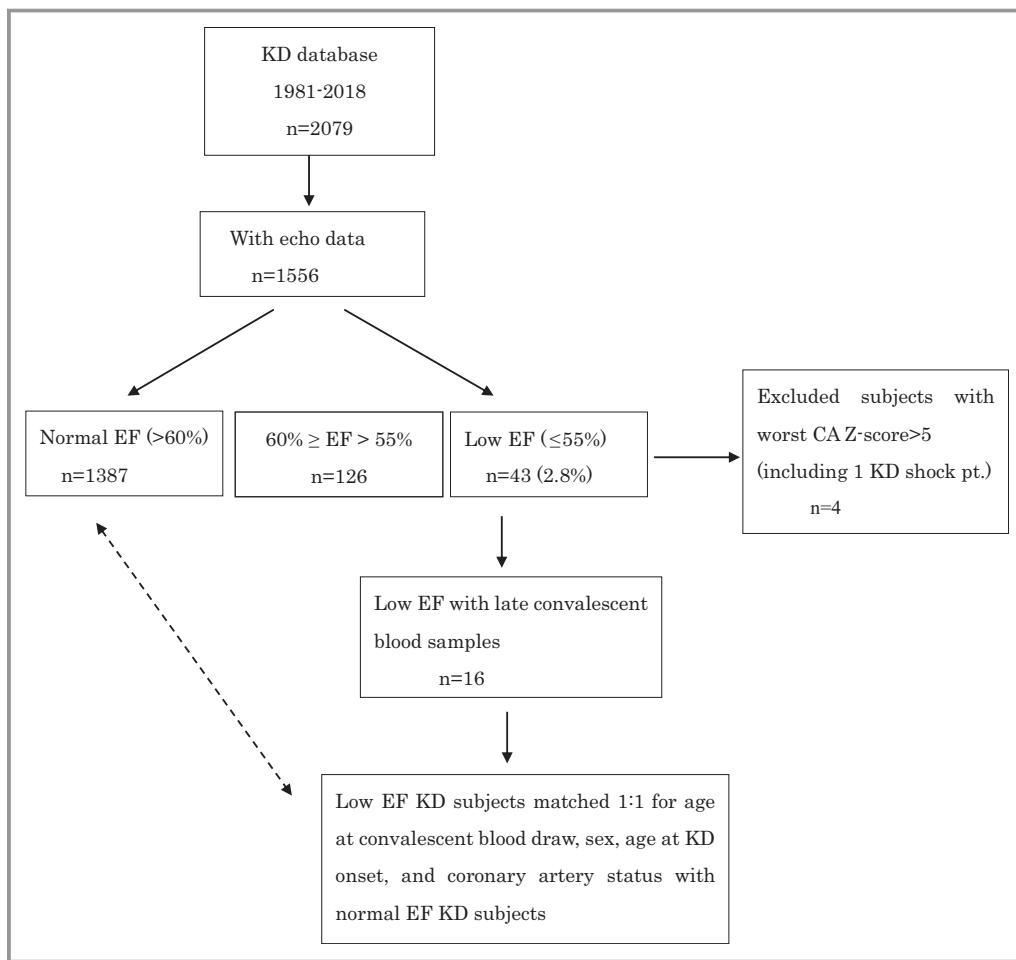
## Study Population

We enrolled all patients with KD with an ejection fraction  $\leq 55\%$  by transthoracic echocardiogram during their acute hospitalization (low EF group) who met American Heart Association (AHA) guidelines for complete or incomplete KD and were diagnosed and treated at Rady Children's Hospital San Diego between March 1981 and Oct 2018. EF was measured during the acute illness at the time of diagnosis by the Teichholz method. Patients  $<3$  years were sedated for the echocardiogram to ensure accurate measurements. Because KD patients with giant aneurysms are known to have elevated levels of biomarkers for inflammation, we chose for further study only those patients whose worst coronary artery z score was  $\leq 5$  SD units.<sup>17,18,20</sup> The worst coronary artery z score was defined as the largest internal diameter during the first year after fever onset of the right coronary artery and left anterior descending coronary artery normalized for body surface area and expressed as SD units from the mean as previously described.<sup>22</sup> From our database and biorepository of KD patient blood samples, we matched the low EF group for sex, age of onset ( $\pm 2$  years), age at sample collection ( $\pm 2$  years), and coronary artery z score ( $\leq 2.0$  or  $>2.0$ ) with KD patients with an EF of  $>60\%$  (Figure 1). For 2 pairs, the age difference was 4.2 years and 2.3 years, respectively. If multiple matches were available, we chose the patient whose onset date was closest to the low EF patient. The study was reviewed and approved by the Institutional Review Board at the University of California San Diego and parents and subjects signed informed consent or assent documents as appropriate.

KD shock syndrome was defined on the basis of systolic hypotension for age, a sustained decrease in systolic blood pressure from baseline of  $\geq 20\%$ , or clinical signs of poor perfusion, as previously reported.<sup>9</sup> IVIG resistance was defined as persistence of fever (oral or rectal temperature  $\geq 38.0^\circ\text{C}$ ) at least 36 hours after the end of the IVIG infusion.

## Sample Collection and Assays

Blood samples were collected at convalescent time points between 0.9 and 11.3 years after KD onset when all patients were generally healthy. Blood was collected and separated immediately by centrifugation and stored at  $-80^\circ\text{C}$ . We measured EDTA plasma levels of calprotectin, Gal-3, sST2, GDF-15, and serum levels of PIPC by ELISA according to the manufacturer's instructions: Calprotectin, Gal-3, GDF-15: R&D Systems, Minneapolis, MN, USA, sST2: Critical



**Figure 1.** Flow diagram showing low EF study population and matched normal EF KD controls. CA indicates coronary artery; EF, ejection fraction; KD, Kawasaki disease.

Diagnostics, San Diego, CA, USA and PIPC: Quidel, San Diego, CA, USA.

## Statistical Analysis

All authors had full access to all the data in the study and take responsibility for its integrity and the data analysis. Data were analyzed using R software version 3.5.2 (<http://www.r-project.org>) and presented as median and interquartile range. Biomarker levels and interquartile range for low EF group and matched controls were compared by paired t-test.

Separate multivariable linear regression models were fitted for each biomarker measurement as the outcome. The EF group was the main predictor (low EF versus normal EF groups), while models were adjusted for age at onset (in Model 1), age at convalescent phlebotomy (in Model 2), sex and worst z score. The assumptions of linear regression were evaluated.  $P < 0.05$  was considered statistically significant.

## Results

### Characteristics of the Study Population

During the 37-year period of our study, 2079 patients were registered in our database with echocardiographic data available from 1556 patients (74.8%). Of these, the EF was  $\leq 55\%$  in 43 patients (2.8%) during the acute phase. Because of the known association of elevated biomarkers of inflammation with persistent giant CAA, 4 subjects with  $Z_{\text{worst}} > 5.0$  were excluded. Late convalescent blood samples were available for 16 of these 39 patients (41%) with low EF. (Figure 1).

### Clinical and Laboratory Data

There was no significant difference between the low and normal EF groups in the demographic or clinical features of KD (Table 1). In the low and normal EF groups, all but 1 patient in the low EF group was diagnosed with complete KD. In the low EF group, 6 (37.5%) subjects were diagnosed

**Table 1.** Characteristics of Kawasaki Disease Patients With Low ( $\leq 55\%$ ) and Age and Sex Matched Kawasaki Disease Patients With Normal ( $>60\%$ ) Ejection Fraction on Initial Echocardiogram

		Normal EF (EF $>60$ ) (n=16)	Low EF (EF $\leq 55\%$ ) (n=16)	P Value
Characteristic				
Age at onset, y		3.6 (2.4–4.5)	3.7 (2.3–5.4)	0.91
% Males		69	63	0.71
Ethnicity, n (%)				
Asian		3 (19)	3 (19)	0.52
Black		0 (0)	1 (6)	
White		3 (19)	2 (13)	
Hispanic		8 (50)	5 (31)	
More than one race		2 (12)	5 (31)	
Median EF (range)		66 (62–75)	52 (42–55)	...
EF at time of phlebotomy (range)		71.0 (60.6–80.4)	66.5 (60.1–70.3)	0.12
Illness day of echo with lowest EF, median (range)		7.5 (5–10)	7 (4–14)	0.78
Age at sample collection, y		7.2 (4.7–8.5)	7.8 (5.4–11.1)	0.78
Interval between KD onset and blood sample, y		1.8 (1.1–6.0)	1.7 (1.1–6.1)	0.76
KD shock, n (%)		0	6 (37.5)	-
IVIG resistance, n (%)		5 (31.3)	10 (62.5)	0.048*
Zworst score for LAD and RCA, median (range)	Zworst score $<2.5$	1.9 (0.3–2.3) n=6	2.2 (1.4–2.3) n=6	0.79
	$2.5 \leq$ Zworst score $<5$	3.1 (2.5–4.6) n=10	3.3 (2.6–4.7) n=10	0.79
Laboratory data, median (IQR)				
	WBC, $\times 10^3/\mu\text{L}$	13.7 (11.2–16.9)	15.5 (10.3–19.9)	0.47
	% neutrophils	77.0 (67.5–87.0)	80.0 (70.8–85.0)	0.32
	Hemoglobin, g/dL	10.3 (9.75–11.1)	10.1 (9.7–11.2)	0.95
	Platelet, $\times 10^3/\mu\text{L}$	435 (276–506)	282.0 (221.5–352.8)	0.04*
	ALT, IU/L	43 (17–108)	60 (43–140)	0.2
	GGT, IU/L	60 (15–183)	53 (24–78)	0.76
	ESR mm/hr	64 (54–79)	75 (46–136)	0.12
	CRP, mg/dL	8.5 (3.3–18.3)	20.7 (13.6–29.5)	0.008*

Zworst was defined as the largest internal CA diameter during the first year after fever onset of the RCA and LAD normalized for body surface area and expressed as standard deviation units from the mean. ALT indicates alanine aminotransferase; CRP, C-reactive protein; EF, ejection fraction; ESR, erythrocyte sedimentation rate; GGT,  $\gamma$ -glutamyl transpeptidase; IVIG, intravenous immunoglobulin; KD, Kawasaki disease; LAD, left anterior descending coronary artery; NS, not significant; RCA, right coronary artery; WBC, white blood cell.

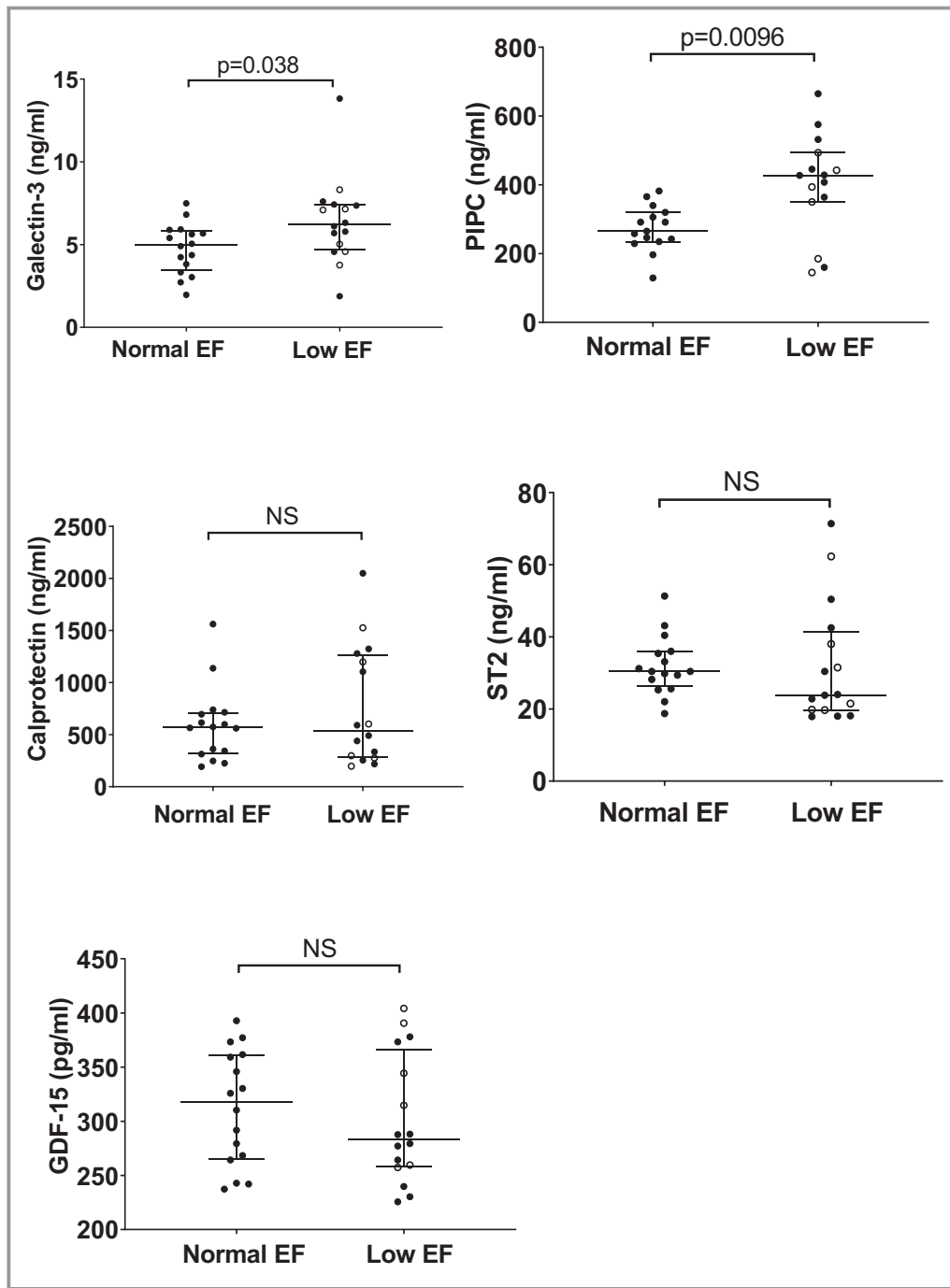
\*Significant *P* values. Continuous values are median and interquartile range unless otherwise noted.

with KD shock syndrome in the acute phase and required pressor support and inotropic agents in addition to standard treatment for KD. The EF normalized to  $>55\%$  in all of the low EF subjects between 2- and 31-days post-treatment (median 6 days, interquartile range 2.0–14.5) and remained normal for the duration of follow-up. IVIG resistance was more common in the low EF group compared with the normal EF group (62.5% and 31.3%, respectively,  $P=0.048$ ). Comparison of pre-treatment laboratory data revealed a higher median C-reactive protein level (20.7 versus 8.5 mg/dL,  $P=0.008$ ) in the low EF group.

## Biomarker Analysis

Plasma levels of Gal-3, calprotectin, sST2 and GDF-15 and serum levels of PIPC were measured in all subjects (Figure 2). In the univariate analysis, the median Gal-3 and PIPC levels in the low EF group were significantly higher than those in the normal EF group (Gal-3: low EF 6.2 versus normal EF 5.0 mg/dL  $P=0.038$ , PIPC: low EF 427.4 versus normal EF 265.2 mg/dL,  $P=0.01$ ). There was no consistent difference in biomarker levels between the KD shock patients and other patients within the low EF group.

Two different models were constructed for the multi-variable analysis. In the first model, EF group (Low EF



**Figure 2.** Comparison of candidate biomarkers of myocardial fibrosis in late convalescent blood samples from low EF and normal EF KD subjects. Bars show median and interquartile range. Open circles: KD shock subjects. EF: ejection fraction; GDF-15, growth-differentiation factor-15; NS, not significant; PIPC, carboxyterminal propeptide of procollagen type I. *P* value by paired *t* test.

versus Normal EF) was the main predictor, adjusting for age at onset, sex, and Zworst. In the second model, EF group was again the main predictor, adjusting for age at time of convalescent phlebotomy, sex, and Zworst. For both models, there was a significant difference between

the Low EF and Normal EF groups for Gal-3 and PIPC. This is consistent with the univariate analysis results (Table 2). Because PIPC levels vary during childhood with linear growth, we assessed the correlation of PIPC levels with age of our subjects at the time of convalescent

**Table 2.** Results of Univariate and Multivariable Analyses of Biomarker Levels in Convalescent Blood Samples From KD Subjects With Normal or Low Ejection Fraction During the Acute Illness

Univariate Analysis (Median [IQR]) (Comparing EF groups)				Multivariable Analysis (Comparing EF groups)					
				Model 1			Model 2		
	Normal EF (>60%)	Low EF (≤55%)	P Value	Estimate	Std. Error	P Value	Estimate	Std. Error	P Value
Galectin-3	4.98 (3.7–5.7)	6.2 (4.9–7.4)	0.038*	−1.64	0.79	0.048*	−1.68	0.79	0.042*
Calprotectin	569.3 (336.6–700.8)	540.9 (292.3–1218.7)	0.3603	−172.71	174.70	0.33	−187.65	168.04	0.27
sST2	30.4 (27.6–35.6)	23.9 (19.8–39.1)	0.982	0.013	4.80	0.998	0.74	4.73	0.88
GDF-15	318.1 (267.3–359.8)	283.5 (258.95–351.5)	0.612	14.52	19.62	0.47	13.93	20.40	0.50
PIPC	265.2 (238.6–312.8)	427.4 (357.1–469.3)	0.0096*	−136.61	43.51	0.004*	−138.38	38.66	0.001*

Levels of Galectin-3 and PIPC were higher in the low EF subjects in the univariate and in both models of the multivariable analysis that corrected for age of onset (Model 1) and age at convalescent phlebotomy (Model 2). \*Significant values. EF indicates ejection fraction; GDF-15, growth-differentiation factor-15; IQR, interquartile range; PIPC, carboxyterminal propeptide of procollagen type I.

phlebotomy, but no significant relationship emerged ( $r^2=0.15$ ).

## Discussion

In this study, we examined biomarkers of cardiovascular inflammation and fibrosis in KD subjects with low EF and normal EF during the acute illness to test the hypothesis that more severe myocarditis during the acute phase of KD could predispose to late myocardial fibrosis. We measured sST2, GDF-15, and PIPC as potential markers of fibrosis, calprotectin as a measure of inflammation, and Gal-3 as a marker of both inflammation and fibrosis. Our study revealed elevated levels of Gal-3 and PIPC but normal levels of calprotectin at least 1 year after KD onset in the low EF subjects suggesting the possibility of sub-clinical myocardial fibrosis.

The biomarkers for this study were specifically chosen to elucidate either myocardial inflammation or fibrosis. The calcium binding proteins S100A8 and S100A9 that form the calprotectin heterodimer are abundant in neutrophils and have long been recognized as markers of inflammation during the acute phase of KD.<sup>23–27</sup> We recently reported that calprotectin levels are elevated in pediatric and adult KD patients with giant CAA years after disease onset.<sup>17</sup> In the present study, there was no significant difference between the normal EF and low EF groups suggesting the absence of persistent inflammation in these patients with normal convalescent coronary artery z scores.

Gal-3, a  $\beta$ -galactoside-binding lectin, has been studied as a potential biomarker for cardiac fibrosis and adverse cardiac remodeling in heart failure.<sup>28,29</sup> Gal-3 is secreted by activated macrophages and is a mediator of both cardiac inflammation and fibrosis. In our study, the median Gal-3 level was increased in the low EF group. Interestingly, Gal-3 has been

detected in  $\alpha$ -smooth muscle actin positive myofibroblasts in the walls of coronary and systemic artery aneurysms and in the myocardium in hearts from adult KD subjects with giant aneurysms.<sup>20</sup> Gal-3 may also be a therapeutic target as demonstrated by an ischemia-reperfusion experiment showing reduced myocardial injury in rats fed modified citrus pectin that inhibits the activity of Gal-3.<sup>30</sup>

GDF-15 belongs to the transforming growth factor-beta (TGF- $\beta$ ) superfamily participates in the regulation of inflammation, immune response, apoptosis and cellular proliferation.<sup>31</sup> GDF-15 is weakly expressed by cardiomyocytes under normal conditions, but is strongly upregulated by inflammatory cytokines and ischemia–reperfusion.<sup>21</sup> In atrial fibrillation and end-stage, non-ischemic dilated cardiomyopathy, the expression of GDF-15 was related to the degree of cardiac fibrosis.<sup>32</sup>

sST2 is a member of the interleukin-1 receptor family and a decoy receptor for interleukin-33, a member of the interleukin-1 family of cytokines whose expression increases under inflammatory conditions.<sup>33</sup> sST2 is released by cardiomyocytes and fibroblasts exposed to myocardial strain.<sup>34</sup> We previously reported that sST2 levels were significantly elevated in acute KD subjects compared with convalescent KD and healthy control subjects,<sup>18</sup> suggesting that sST2 may also be a biomarker of myocardial injury. Some studies suggest that sST2 may have a direct role in fibrosis or remodeling.<sup>35,36</sup> In rat acute myocardial infarction model, cardiomyocyte expression of sST2 was upregulated during the first 4 weeks following acute myocardial infarction and correlated with the ongoing processes of fibrosis and inflammation.<sup>37</sup>

Inflammation and fibrosis are hallmarks of the myocardial injury during KD. Acute samples were no longer available for many of these patients. However, in previously published studies, we reported that acute calprotectin, Gal-3, and sST2



levels were elevated compared with the convalescent phase regardless of EF or coronary artery status.<sup>17,18,20</sup> Autopsies of KD patients with severe myocarditis demonstrate degenerative necrosis of the myocardium associated with marked inflammatory cell infiltration.<sup>38</sup> Myocardial tissues from 29 patients who died within 40 days from KD onset showed inflammatory cell infiltration in the myocardium and 8 of these patients had no evidence of CAA.<sup>38</sup> Histology of endomyocardial biopsies during the acute phase of KD documented universal myocardial inflammation and repeat biopsy >3 years later showed persistence of histopathologic abnormalities in the myocardium in many subjects.<sup>39</sup> However, the subsequent evolution of this myocardial inflammation has largely been addressed through autopsy studies. Severe myocardial fibrosis in young adults following KD in childhood has been noted in case reports of explanted hearts from patients requiring transplant and in small series of autopsy cases.<sup>20,40–42</sup> Histology from autopsies of young adults with giant aneurysms from KD showed diffuse, bridging fibrosis that was not confined to the territories supplied by the affected coronary arteries.<sup>42</sup>

Imaging studies with cardiac MRI (CMRI) have attempted to document myocardial fibrosis in convalescent KD patients with late gadolinium enhancement. To evaluate the extent of global cardiac fibrosis by non-invasive methods, several studies have focused on post-contrast CMRI myocardial T1 time to quantify diffuse myocardial fibrosis,<sup>43,44</sup> but the application of these techniques to a KD population has largely failed to demonstrate abnormalities in the absence of known ischemic events.<sup>45–47</sup> Analysis of CMRI in KD patients with small to giant aneurysms using a segmental approach identified regional fibrosis that was related to decreased myocardial perfusion.<sup>48</sup> The use of CMRI to perform feature tracking demonstrated abnormal longitudinal and circumferential strain in convalescent KD patients regardless of coronary artery status.<sup>46</sup> As an alternative approach, a study of 90 Han Chinese KD patients demonstrated abnormal values of calibrated integrated backscatter by echocardiography as evidence of myocardial fibrosis irrespective of coronary artery status.<sup>49</sup> Additional evidence for susceptibility to myocardial fibrosis comes from the *Lactobacillus casei* cell wall extract murine model of KD in which isoproterenol chemical stress induced myocardial fibrosis only in *Lactobacillus casei* cell wall extract-exposed mice.<sup>50</sup>

We recognize both strengths and limitations of our study. This is the first study to focus on myocardial outcomes as opposed to coronary artery outcomes in KD patients who presented with low EF in the initial phase of the disease. The results raise concerns about the current practice of discharging KD patients without aneurysms from care following the 4-week visit as outlined in the current AHA guidelines, which would have applied to 6 patients in our low EF group.<sup>2</sup> The

limitations of the study include the cross-sectional design with a small sample size and as such, the study should be viewed as exploratory. Validation is required with longitudinal data from the same patients over time to understand if the process of myocardial fibrosis is progressive or static. In addition, imaging studies including CMRI with T1 mapping should be included to provide further evidence of subclinical myocardial fibrosis. Measurement of the myocardial perfusion reserve index coupled with the biomarker analysis could help to delineate if fibrosis is related to microvascular insufficiency as has been suggested by previous studies.<sup>45,51</sup> If findings from this study are validated by imaging, therapeutic intervention to prevent future myocardial fibrosis may be warranted.

## Conclusions

KD patients presenting with isolated low EF with normal coronary artery dimensions during the acute phase of their illness may be at risk for myocardial fibrosis as a late sequela. Further studies are needed to determine the dynamics of these biomarker levels over time and to link these data to imaging studies of structural changes in the myocardium. This study identifies a subset of KD patients who may warrant additional surveillance in the late convalescent period, although this is not a recommendation in the current AHA guidelines.

## Sources of Funding

This work supported in part by a grant from the Gordon and Marilyn Macklin Foundation to Burns and a grant from the Kawasaki Disease Foundation to Burns.

## Disclosures

None.

## References

1. Newburger JW, Takahashi M, Burns JC. Kawasaki disease. *J Am Coll Cardiol*. 2016;67:1738–1749.
2. McCrindle BW, Rowley AH, Newburger JW, Burns JC, Bolger AF, Gewitz M, Baker AL, Jackson MA, Takahashi M, Shah PB, Kobayashi T, Wu MH, Saji TT, Pahl E. Diagnosis, treatment, and long-term management of Kawasaki disease: a scientific statement for health professionals from the American Heart Association. *Circulation*. 2017;135:e927–e999.
3. Gordon JB, Daniels LB, Kahn AM, Jimenez-Fernandez S, Vejar M, Numano F, Burns JC. The spectrum of cardiovascular lesions requiring intervention in adults after Kawasaki disease. *JACC Cardiovasc Interv*. 2016;9:687–696.
4. Kao CH, Hsieh KS, Wang YL, Chen CW, Liao SQ, Wang SJ, Yeh SH. Tc-99 m HMPAO WBC imaging to detect carditis and to evaluate the results of high-dose gamma globulin treatment in Kawasaki disease. *Clin Nucl Med*. 1992;17:623–626.
5. Matsuura H, Ishikita T, Yamamoto S, Umezawa T, Ito R, Hashiguchi R, Saji T, Matsuo N, Takano M. Gallium-67 myocardial imaging for the detection of myocarditis in the acute phase of Kawasaki disease (mucocutaneous lymph node syndrome): the usefulness of single photon emission computed tomography. *Br Heart J*. 1987;58:385–392.



6. Hiraishi S, Yashiro K, Oguchi K, Kusano S, Ishii K, Nakazawa K. Clinical course of cardiovascular involvement in the mucocutaneous lymph node syndrome. Relation between clinical signs of carditis and development of coronary arterial aneurysm. *Am J Cardiol*. 1981;47:323–330.
7. Moran AM, Newburger JW, Sanders SP, Parness IA, Spevak PJ, Burns JC, Colan SD. Abnormal myocardial mechanics in Kawasaki disease: rapid response to gamma-globulin. *Am Heart J*. 2000;139:217–223.
8. Anderson TM, Meyer RA, Kaplan S. Long-term echocardiographic evaluation of cardiac size and function in patients with Kawasaki disease. *Am Heart J*. 1985;110:107–115.
9. Kanegaye JT, Wilder MS, Molkara D, Frazer JR, Pancheri J, Tremoulet AH, Watson VE, Best BM, Burns JC. Recognition of a Kawasaki disease shock syndrome. *Pediatrics*. 2009;123:e783–e789.
10. Dominguez SR, Friedman K, Seewald R, Anderson MS, Willis L, Glode MP. Kawasaki disease in a pediatric intensive care unit: a case-control study. *Pediatrics*. 2008;122:e786–e790.
11. Yutani C, Go S, Kamiya T, Hirose O, Misawa H, Maeda H, Kozuka T, Onishi S. Cardiac biopsy of Kawasaki disease. *Arch Pathol Lab Med*. 1981;105:470–473.
12. Yonesaka S, Takahashi T, Eto S, Sato T, Otani K, Ueda T, Sato A, Kitagawa Y, Konno Y, Kinjo M. Biopsy-proven myocardial sequelae in Kawasaki disease with giant coronary aneurysms. *Cardiol Young*. 2010;20:602–609.
13. Januzzi JL Jr, van Kimmenade RR. Importance of rigorous evaluation in comparative biomarker studies. *J Am Coll Cardiol*. 2014;63:167–169.
14. Lopez B, Gonzalez A, Ravassa S, Beaumont J, Moreno MU, San Jose G, Querejeta R, Diez J. Circulating biomarkers of myocardial fibrosis: the need for a reappraisal. *J Am Coll Cardiol*. 2015;65:2449–2456.
15. Poulsen SH, Host NB, Egstrup K. Long-term changes in collagen formation expressed by serum carboxyterminal propeptide of type-I procollagen and relation to left ventricular function after acute myocardial infarction. *Cardiology*. 2001;96:45–50.
16. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: Insights from the randomized aldactone evaluation study (rales). Rales investigators. *Circulation*. 2000;102:2700–2706.
17. Lech M, Guess J, Duffner J, Oyama J, Shimizu C, Hoshino S, Farutin V, Bulik DA, Gutierrez B, Sarvaia H, Kapoor B, Koppes L, Saldova R, Stockmann H, Albrecht S, McManus C, Rudd PM, Kaundinya GV, Manning AM, Bosques CJ, Kahn AM, Daniels LB, Gordon JB, Tremoulet AH, Capila I, Gunay NS, Ling LE, Burns JC. Circulating markers of inflammation persist in children and adults with giant aneurysms after Kawasaki disease. *Circ Genom Precis Med*. 2019;12:e002433.
18. Sato YZ, Molkara DP, Daniels LB, Tremoulet AH, Shimizu C, Kanegaye JT, Best BM, Snider JV, Frazer JR, Maisel A, Burns JC. Cardiovascular biomarkers in acute Kawasaki disease. *Int J Cardiol*. 2013;164:58–63.
19. Dumić J, Dabelić S, Flogel M. Galectin-3: an open-ended story. *Biochim Biophys Acta*. 2006;1760:616–635.
20. Numano F, Shimizu C, Jimenez-Fernandez S, Vejar M, Oharaseki T, Takahashi K, Salgado A, Tremoulet AH, Gordon JB, Burns JC, Daniels LB. Galectin-3 is a marker of myocardial and vascular fibrosis in Kawasaki disease patients with giant aneurysms. *Int J Cardiol*. 2015;201:429–437.
21. Kempf T, Eden M, Strelau J, Naguib M, Willenbockel C, Tongers J, Heineke J, Kotlarz D, Xu J, Molkentin JD, Niessen HW, Drexler H, Wollert KC. The transforming growth factor-beta superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. *Circ Res*. 2006;98:351–360.
22. Dallaire F, Dahdah N. New equations and a critical appraisal of coronary artery z scores in healthy children. *J Am Soc Echocardiogr*. 2011;24:60–74.
23. Wittkowski H, Hirono K, Ichida F, Vogl T, Ye F, Yanlin X, Saito K, Uese K, Miyawaki T, Viemann D, Roth J, Foell D. Acute Kawasaki disease is associated with reverse regulation of soluble receptor for advance glycation end products and its proinflammatory ligand S100A12. *Arthritis Rheum*. 2007;56:4174–4181.
24. Hirono K, Foell D, Xing Y, Miyagawa-Tomita S, Ye F, Ahlmann M, Vogl T, Futatani T, Rui C, Yu X, Watanabe K, Watanabe S, Tsubata S, Uese K, Hashimoto I, Ichida F, Nakazawa M, Roth J, Miyawaki T. Expression of myeloid-related protein-8 and -14 in patients with acute Kawasaki disease. *J Am Coll Cardiol*. 2006;48:1257–1264.
25. Ebihara T, Endo R, Kikuta H, Ishiguro N, Ma X, Shimazu M, Otoguro T, Kobayashi K. Differential gene expression of s100 protein family in leukocytes from patients with Kawasaki disease. *Eur J Pediatr*. 2005;164:427–431.
26. Ye F, Foell D, Hirono KI, Vogl T, Rui C, Yu X, Watanabe S, Watanabe K, Uese K, Hashimoto I, Roth J, Ichida F, Miyawaki T. Neutrophil-derived S100A12 is profoundly upregulated in the early stage of acute Kawasaki disease. *Am J Cardiol*. 2004;94:840–844.
27. Foell D, Ichida F, Vogl T, Yu X, Chen R, Miyawaki T, Sorg C, Roth J. S100A12 (EN-RAGE) in monitoring Kawasaki disease. *Lancet*. 2003;361:1270–1272.
28. Heymans S, Gonzalez A, Pizard A, Papageorgiou AP, Lopez-Andres N, Jaisser F, Thum T, Zannad F, Diez J. Searching for new mechanisms of myocardial fibrosis with diagnostic and/or therapeutic potential. *Eur J Heart Fail*. 2015;17:764–771.
29. Rabinovich GA, Toscano MA. Turning ‘sweet’ on immunity: galectin-glycan interactions in immune tolerance and inflammation. *Nat Rev Immunol*. 2009;9:338–352.
30. Ibarrola J, Matilla L, Martinez-Martinez E, Guertel A, Fernandez-Celis A, Henry JP, Nicol L, Jaisser F, Mulder P, Ouyard-Pascaud A, Lopez-Andres N. Myocardial injury after ischemia/reperfusion is attenuated by pharmacological galectin-3 inhibition. *Sci Rep*. 2019;9:9607.
31. Goletti S, Gruson D. Personalized risk assessment of heart failure patients: more perspectives from transforming growth factor super-family members. *Clin Chim Acta*. 2015;443:94–99.
32. Lok SI, Winkens B, Goldschmeding R, van Geffen AJ, Nous FM, van Kuik J, van der Weide P, Kloppe C, Kirkels JH, Lahpor JR, Doevendans PA, de Jonge N, de Weger RA. Circulating growth differentiation factor-15 correlates with myocardial fibrosis in patients with non-ischaemic dilated cardiomyopathy and decreases rapidly after left ventricular assist device support. *Eur J Heart Fail*. 2012;14:1249–1256.
33. Schmitz J, Owyang A, Oldham E, Song Y, Murphy E, McClanahan TK, Zurawski G, Moshrefi M, Qin J, Li X, Gorman DM, Bazan JF, Kastelein RA. IL-33, an interleukin-1-like cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*. 2005;23:479–490.
34. Kakkar R, Lee RT. The IL-33/ST2 pathway: therapeutic target and novel biomarker. *Nat Rev Drug Discov*. 2008;7:827–840.
35. Weir RA, Miller AM, Murphy GE, Clements S, Steedman T, Connell JM, McInnes IB, Dargie HJ, McMurray JJ. Serum soluble ST2: a potential novel mediator in left ventricular and infarct remodeling after acute myocardial infarction. *J Am Coll Cardiol*. 2010;55:243–250.
36. Michalska-Kasieczak M, Bielecka-Dabrowa A, von Haehling S, Anker SD, Rysz J, Banach M. Biomarkers, myocardial fibrosis and co-morbidities in heart failure with preserved ejection fraction: an overview. *Arch Med Sci*. 2018;14:890–909.
37. Sanchez-Mas J, Lax A, Asensio-Lopez Mdel C, Fernandez-Del Palacio MJ, Caballero L, Santarelli G, Januzzi JL, Pascual-Figal DA. Modulation of IL-33/ST2 system in postinfarction heart failure: correlation with cardiac remodelling markers. *Eur J Clin Invest*. 2014;44:643–651.
38. Harada M, Yokouchi Y, Oharaseki T, Matsui K, Tobayama H, Tanaka N, Akimoto K, Takahashi K, Kishiro M, Shimizu T, Takahashi K. Histopathological characteristics of myocarditis in acute-phase Kawasaki disease. *Histopathology*. 2012;61:1156–1167.
39. Yonesaka S, Takahashi T, Matubara T, Nakada T, Furukawa H, Tomimoto K, Oura H. Histopathological study on Kawasaki disease with special reference to the relation between the myocardial sequelae and regional wall motion abnormalities of the left ventricle. *Jpn Circ J*. 1992;56:352–358.
40. Orenstein JM, Shulman ST, Fox LM, Baker SC, Takahashi M, Bhatti TR, Russo PA, Mierau GW, de Chadarevian JP, Perlman EJ, Trevenen C, Rotta AT, Kalelkar MB, Rowley AH. Three linked vasculopathic processes characterize Kawasaki disease: a light and transmission electron microscopic study. *PLoS One*. 2012;7:e38998.
41. Murai T, Kuroda N, Shinozuka T, Kurihara K, Yanagida J, Watanabe H, Saito K, Muraishi M, Imai M. Coronary aneurysms in a young adult: report of a case suspected of Kawasaki disease. *Med Sci Law*. 1989;29:59–63.
42. Shimizu C, Sood A, Lau HD, Oharaseki T, Takahashi K, Krous HF, Campman S, Burns JC. Cardiovascular pathology in 2 young adults with sudden, unexpected death due to coronary aneurysms from Kawasaki disease in childhood. *Cardiovasc Pathol*. 2015;24:310–316.
43. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta SN, Kaye DM, Taylor AJ. Evaluation of diffuse myocardial fibrosis in heart failure with cardiac magnetic resonance contrast-enhanced T1 mapping. *J Am Coll Cardiol*. 2008;52:1574–1580.
44. Mascherbauer J, Marzluft BA, Tufaro C, Pfaffenberger S, Graf A, Wexberg P, Panzenbock A, Jakowitsch J, Bangert C, Laimer D, Schreiber C, Karakus G, Hulsman M, Pacher R, Lang IM, Maurer G, Bonderman D. Cardiac magnetic resonance postcontrast T1 time is associated with outcome in patients with heart failure and preserved ejection fraction. *Circ Cardiovasc Imaging*. 2013;6:1056–1065.
45. Bratis K, Chiribiri A, Hussain T, Krasemann T, Henningson M, Phinikaridou A, Mavrogeni S, Botnar R, Nagel E, Razavi R, Greil G. Abnormal myocardial perfusion in Kawasaki disease convalescence. *JACC Cardiovasc Imaging*. 2015;8:106–108.

46. Bratis K, Hachmann P, Child N, Krasemann T, Hussain T, Mavrogeni S, Botnar R, Razavi R, Greil G. Cardiac magnetic resonance feature tracking in Kawasaki disease convalescence. *Ann Pediatr Cardiol.* 2017;10:18–25.
47. Tacke CE, Romeih S, Kuipers IM, Spijkerboer AM, Groenink M, Kuijpers TW. Evaluation of cardiac function by magnetic resonance imaging during the follow-up of patients with Kawasaki disease. *Circ Cardiovasc Imaging.* 2013;6:67–73.
48. Muthusami P, Luining W, McCrindle B, van der Geest R, Riesenkampff E, Yoo SJ, Seed M, Manlhiot C, Grosse-Wortmann L. Myocardial perfusion, fibrosis, and contractility in children with Kawasaki disease. *JACC Cardiovasc Imaging.* 2018;11:1922–1924.
49. Xie L, Wang R, Huang M, Zhang Y, Shen J, Xiao T. Quantitative evaluation of myocardial fibrosis by cardiac integrated backscatter analysis in Kawasaki disease. *Cardiovasc Ultrasound.* 2016;14:3.
50. Matundan HH, Sin J, Rivas MN, Fishbein MC, Lehman TJ, Chen S, Gottlieb RA, Crother TR, Abe M, Arditi M. Myocardial fibrosis after adrenergic stimulation as a long-term sequela in a mouse model of Kawasaki disease vasculitis. *JCI insight.* 2019;4:126279.
51. Friesen RM, Schafer M, Jone PN, Appiawiah N, Vargas D, Fonseca B, DiMaria MV, Truong U, Malone L, Browne LP. Myocardial perfusion reserve index in children with Kawasaki disease. *J Magn Reson Imaging.* 2018;48:132–139.